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Gas Exchange and Yields of Bt Resistant Maize (Zea mays L.) with European Corn Borer (Ostrinia nubilalis, Hubner) Infestation

Shad Mallady
Eastern Illinois University

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Gas Exchange and Yields of Bt Resistant Maize (Zea mays L.)

with European Corn Borer (Ostrinia nubilalis, Hubner) Infestation

(TITLE)

BY

Shad Mallady

THESIS

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Abstract

Seed companies have transgenic maize (*Zea mays* L.) hybrids resistant to European corn borer (ECB) [*Ostrinia nubilalis* (Hübner)]. However, the impact of this *Bt* (*Bacillus thuringiensis*) gene on other plant processes is not understood. In 1997 and 1999, a study at Central Golden Harvest Research in Clinton, IL focused on how the *Bt* gene affects gas exchange and yields of maize with and without ECB infestation. *Bt* and non-*Bt* isogenic pairs were planted with or without nets and/or insecticides to eliminate natural infestation of ECB, and with or without manual infestation of ECB. Photosynthesis and transpiration were measured. At harvest, yields and kernel mass were determined. Seed germination percentages and root lengths were measured. Photosynthesis and transpiration were similar for *Bt* and non-*Bt* plants regardless of ECB infestation. In 1997 and 1999, yields were not significantly different for *Bt* and non-*Bt* plants, but in 1999 yields were significantly higher in plants with nets or insecticides compared to no nets or insecticides. In 1997, kernel mass was significantly higher for *Bt* than non-*Bt* plants (28.6 and 26.7 g, respectively). In 1999, kernel mass was not significantly different between *Bt* and non-*Bt* plants; however one isogenic pair of hybrids, plants with insecticide had significantly larger kernels and more seed produced than those without insecticide. Seed vigor showed no significant differences for germination percentages or root length. Thus, *Bt* genes had no significant effects on gas exchange or yields regardless of ECB infestation, but had a significant effect on kernel mass in 1997.

Dedication Page

Dr. Janice M. Coons – Department of Biological Sciences, Eastern Illinois University, Charleston, IL 61920.

Dr. Judy A. Damery-Parrish – Department of Science, Millikin University, Decatur, IL 62522.

Dr. Kevin T. Montgomery - Central Golden Harvest Research, Clinton, IL 61727.

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Introduction

European corn borer (ECB), *Ostrinia nubilalis* (Hübner), is a pest causing great damage to maize hybrids in the United States Corn Belt. ECB is responsible for annual maize production losses of several millions of dollars in the northern Corn Belt (Barry and Darrah 1991, Russnogle 1997, Lauer and Wedberg 1999). The insect is an introduced species that arrived in the early 1900s in broomcorn from Hungary and Italy (Siegel et al. 1987). Corn borers first were established near Boston, MA in 1917 with a single generation life cycle per year. Once the European corn borer reached Illinois in 1939, it had established a two-generation cycle. As the insect became established in the southern states, the ECB developed third and fourth generation per year cycles due to a longer growing period and warmer climates. Many plants are susceptible to corn borer damage including maize, sorghum, cotton and many vegetables (Mason et al. 1996). For years scientists have tried to quantify the relationship between infestation level and corn yield reduction (Calvin et al. 1988). Chaing et al. (1954) showed that second generation borer feeding had little effect on ear growth but was responsible for stalk breakage and ear drop. Yet in 1960, Chaing et al. indicated that the first generation was a better indicator of yield reductions in field corn.

The European corn borer belongs to the Family Pyralidae in the Order Lepidoptera. Its life cycle consists of the adult female laying eggs on the under surface of the leaf, where the eggs hatch and the developing borers move into the whorl of the developing plant. The first and second instars, of the five-instar life cycle, occur inside the whorl of the plant where the larvae feed on the leaves and use the plant for protection. Reaching the third instar of the life cycle, the ECB larvae begin to bore into the stalk of the corn plants. They remain inside eating the vascular tissue and developing until they pupate. Transition insects such as boring insects may cause physiological problems by decreasing the photosynthetic rate, and therefore slowing the productivity of the plant (Mason et al. 1996).

One method to combat the destructive behavior of this pest was the insertion of a soil-bacterium, *Bacillus thuringiensis* (*Bt*) into the corn genome. The inserted bacterium (*Bt*) allows the plant to produce a *Cry* protein. This protein, when ingested after eating stems and leaves, causes the digestive system of the ECB to become inactive. Many maize hybrids have been engineered to incorporate *Bt* only in the leaf and stalk tissues and not in the kernels or pollen (Mason et al. 1996). The protein toxin in the leaf and stalk is an effective control for the first and second generation of the borer (Mason et al. 1996, Traore et al. 2000). A benefit of genetic engineering of maize is the inhibition of the development of ECB, but it also may allow farmers to decrease the amounts of pesticide applied to the field, a beneficial environmental practice.

What effect the *Bt* bacterium has on the maize plant through physiological factors such as gas exchange, water movement or ion exchange in the plant is not entirely understood. The toxic bacterium decreases the damage due to infestation of the European corn borer, but whether a cost occurs as decreased yields, inhibited photosynthesis and transpiration, reduced rates of growth and development, or lower seed vigor is unclear. European corn borer feeding on vascular tissues can influence grain weight by interfering with photosynthate assimilation and movement to the grain (Calvin et al. 1988).

Many of the previous studies relating to European corn borer infestation in maize yields were conducted before 1960. These studies (Chaing and Hodson 1950) and more recent ones (Lynch 1980, Lynch et al. 1980, Raemisch and Walgenbach 1984 and Umeozor et al. 1985) focus only on maize yield data. It is difficult to find studies focused on the effects of the *Bt* trait on physiological factors that occur with the maize plant.

The focus of this study is on the effects of *Bt* bacteria incorporation in plants to determine if there is a physiological cost or loss in yield to the plant expressing the bacterial gene. Differences between *Bt* hybrids and their non-transgenic counterparts were compared for photosynthesis and transpiration levels, kernel totals, kernel weights, and seed vigor (from germination and root lengths). These parameters were compared for *Bt* and non-*Bt* hybrids under several treatment variables. These treatments consisted of European corn borer infestation over a two-generation life cycle, the use of nets covering the corn and/or the application of an insecticide to prevent natural ECB infestation.

Materials and Methods

Three isogenic pairs of *Zea mays* (L.) hybrids were used: H-2493 and H-2493*Bt* (1997); H-2581 and H-9481*Bt* (1999); and H-9345 and 999803*Bt* (1999) with maturation times of 109, 114 and 114 days, respectively. Choices of hybrid pairs in each year were dependent upon the seed availability from seed banks. Each pair contains a transgenic (resistant) and non-transgenic (susceptible) line. The transgenic hybrids contain the *Bacillus thuringiensis* (*Bt*) bacterium. Hybrids were planted at Central Golden Harvest Research, Clinton, IL on May 22, 1997 the second year's study on May 26, 1999. The plots were planted with a Kinze 4 row cone plot planter (Almaco, Nevada, IA). Plots were 4.6 m long and 0.8 m apart, Seeds were placed at a depth of 5 cm with 25 seeds per row and thinned to 20 plants per row at the five-leaf stage. Each whole plot consisted of all the treatments and each plot was a treatment-hybrid combination consisting of a single row for an area of 3.7 m^2. There were 4 replications per treatment in 1999 and 5 replications for 1997 in the field study. The seed was planted in a Sable soil which is a poorly drained soil in swales and on flats in the uplands (Windhorn 1991). Both years, in mid-April before planting, nitrogen was added to the soil as a 28% liquid solution (by weight), that was a mixture of ammonium nitrate and urea, marketed commercially as URAN. During the two years of the study, pre-plant and post-emergence herbicides were applied to the fields. Dual II (pre-plant) and Atrex 4L (post-emergence) were applied in 1997 at the recommended rate. Dual II (pre-plant) and Spirit (post-emergence) herbicides were applied in 1999. In 1999, an additional insecticide, Lorsban diluted at 300 ml hectare was applied for control of rootworm beetles near mid-flowering. Environmental parameters for heat units and precipitation (Figs. 1 and 2) were recorded at the Golden Harvest weather station (GHWS) in 1997 and 1999 except for March 16 - August 31, 1999 when parameters were recorded at Lincoln, IL weather station due to a lightning strike at the Golden Harvest weather station.

The first year (1997) and second year (1999) consisted of a stratified random arrangement of the hybrids. In 1997 the plots were arranged where the hybrids were either infested with European corn borer (ECB) or sprayed with a pesticide to stop the natural infestation. In 1999, the experimental design was changed to incorporate the use of nets covering the plants within the replications. Nets were used as a deterrent for the natural infestation of corn borer in case pesticides had adverse effects. However, the nets developed large holes due to environmental stress from wind and rain, and were unusable, so pesticides were used again starting at the end of July. Hence, the four treatments consisted of: Nets or insecticide/Infest ECB, Nets or insecticide/No ECB, No nets or insecticide/Infest ECB, and No nets or insecticide/No ECB. Experimental plots utilizing infested insects were separated from the control plots with two rows of border plants to decrease the migration of European corn borer into non-infested treatment plots. To control *ECB*, plots were sprayed with an insecticide, *Pounce* (1997) or *Capture* (1999) (10% solution with 100 ml water) once a week using a hand held sprayer misting above the canopy to decrease corn borer infestation. The plants in the experimental plots where insects were added, were infested with two separate 1st instar generations of European corn borer during the early whorl stage (late June) and on the shank of the ear early August during both years. The European corn borer egg masses were provided by Garst Seed Company Inc., (Slater IA). A mixture of finely ground corn cob grits (through 40 mesh screen, The Anderson, Maumee, OH) and newly hatched corn borer larvae were added to a plastic bottle. Larvae were dispensed using handheld spring-loaded bazooka (Country Plastics, Ames, IA) to release one cubic centimeter =1 "shot" of the mixture onto the plant. Each shot averaged 40 to 60 corn borers. Two shots were applied per plant for each infestation period, therefore allowing for heavy infestation on the maize.

Photosynthesis and transpiration rates were measured approximately every two weeks beginning June 27, 1997 and July 23, 1999 and ending in early September. The LCA-4, Analytical Development Company Infrared Gas Analysis Leaf Chamber Analyzer (Houston, TX) was used to measure photosynthesis and transpiration. The sixth plant of every row (1997) and three random plants per row (1999) as determined by a random numbers table were used. Leaves used with the LCA-4 were located at the top node with a fully expanded leaf of the plant during vegetative growth and one node above the ear during the reproductive plant growth. Each measurement took 45 seconds to complete, and measurements began during the peak sunlight period starting at 10:00 am and ending as late as 5:00 PM. All LCA-4 sessions began the same, with the first replication.

Harvest included the removal of the first 10 ears from each row in 1997, and all ears in 1999. We determined the seed was ready for harvest by first noticing a physiological maturity of the plant and also checking for the blackened abscission layer on the kernel. A change of color for the abscission layer indicates the end of active transport so no dry matter is accumulating in the kernel. Seed was removed from the cob and counted using one of two light beam seed counters, i.e. FMC Corp., Seed Burro 801, Homer City, PA (1997) and International Marketing and Design Corp., Old Mill #900, San Antonio, TX (1999). Total number of seed was counted for each plot in 1997 and in 1999. Ten (1997) and eight (1999) samples of 100 kernels per row were used in seed weight studies. Kernel mass was taken after drying to determine the dry weight of the seed. The seed was dried at a temperature of 40°C for 10 days.

One mixed sample of kernels per row were used to determine germination percentages (both years) and root lengths (1999). The ragdoll method was used in the greenhouse (1997) and the Percival Scientific Inc. (Boone, IA) seed germinator (1999). In 1997, five ragdolls (rolled paper towels, Envision Acclaim, Fort James, Deerfield, IL) of 20 kernels each taken from each plot were placed on a plastic plant tray (52 x 25x 7cm) and put under moist conditions for seven days. The number of germinated seed was recorded. For the 1999 germination tests, a series of 4 replications was conducted, each lasting for 7 days. Each ragdoll contained 20 kernels with 5 kernels from each hybrid from each of four reps within the same treatment (*i.e. nets or insecticide/infest, nets or insecticide/no ECB etc).* Sixteen (4 hybrids x 2 nets or insecticides x 2 infestations) ragdolls were used for each of the four replications. The germinator was set for a 16-hour day at a temperature of 25°C. Upon completion of the incubation period, germinated seed was counted and root lengths were measured.

The 1999 data were analyzed by 3-way analysis of variance (ANOVA). Sources of variation were corn hybrids, use of nets/insecticides, and the infestation of European corn borer. A separate ANOVA test was used for each isogenic pair. For 1997 data, a 2-way ANOVA (no nets) was used incorporating corn hybrids and infestation of corn borers. The statistical program, Co-Stat (1986), was used. The Duncan's multiple range test was used to separate means at a significance level of $P \leq 0.05$.

Results and Discussion

Photosynthesis and transpiration rates for 1997 and 1999 showed no significant differences between the isogenic maize pairs any time over the season (Fig. 3). In 1997 the photosynthesis and transpiration rates followed a gradual curve from high levels during early growth to lower levels as the plants matured. These results were different for 1999, showing an approximate 25 $\mu mol\ m^{-2}\ s^{-1}$ difference for photosynthesis and 5 $mol\ m^{-2}\ s^{-1}$ difference for transpiration during late July between years. However, as the plants matured, rates balanced between the two seasons. Environmental parameters may have hindered the photosynthesis and transpiration levels in the beginning months of the 1999 season. As shown in Fig 1 and 2, high temperatures and low rainfall in late June and most of July would cause the plants to conserve water therefore decreasing its photosynthesizing capabilities.

In 1999, a block effect occurred (results not shown) for both photosynthesis and transpiration rates within the replications, being highest in replication 1 and lowest in replication 4. This effect possibly was due to changes in light levels over the day. All of the photosynthesis and transpiration measurements began at the same time (10:00 AM) during both years. In 1997 only one plant was measured per plot in each treatment over all replications, and measurements were completed by 2:00 PM (i.e. during peak sunlight of the day) which could account for active gas exchange in plants throughout the sampling time. In contrast, in 1999, three plants were measured per treatment in each plot over all replications, which tripled time required for data collection.

Measurements did not conclude until 5:00 PM (i.e. past the peak sunlight of the day) which could account for lower gas exchange near the end of the sampling time.

Other factors were the use of nets/insecticides to exclude insects or the manual infestation of European corn borer to the plants of which neither showed significant differences in gas exchange (data not shown). According to Godfrey et al. (1991) in a 2-year field study, European corn borer larval tunneling in 1987 significantly reduced corn photosynthetic rates by 11.4 and 22.1% with 3 and 5 larvae, respectively per plant, whereas 1 larvae per plant infestation significantly increased photosynthetic rate. These data did not correlate with our data where no significant differences in photosynthesis between infested or non-infested hybrids were seen. Other studies show that with heavy infestation of ECB to the corn plants, higher photosynthetic and lower transpiration rates in the non-transgenic hybrids might occur, considering the plant responses to the insect stress by producing more sugars to compensate for the damage done to xylem and other tissues by boring insects (Calvin 1988).

Seed totals for 1997 hybrids showed no significant difference between the members of an isogenic pair (H-2493 vs. H-2493*Bt*), or any interaction between the hybrids and the use of insecticides or the infestation of European corn borer (Table 1). In 1999, no significant difference was found between hybrids for seed totals for the members of the isogenic pair (H-2581 vs. H-9481*Bt*) or (H-9345 vs. 999803*Bt*) although plants within treatments using nets/insecticides to exclude

European corn borer from the plots had higher seed totals than plants without the nets/insecticides (Table 2).

In 1997, seed mass of *Bt* and non-*Bt* isogenic pairs were significantly different with an increased mass for *Bt* plants (Table 1). No significant difference was found between treatments with insecticides or with ECB infestation. In 1999, the isogenic pair H-2581 and H-9481*Bt* showed a significant difference for seed mass. Seed mass was higher for plots treated with nets/insecticides than for non-treated plots (Table 2). For the second isogenic pair, H-9345 and 999803*Bt*, no significant differences occurred between any of the factors.

Differences in seed vigor for *Bt* isogenic pairs were estimated by utilizing germination percentages and root lengths (Table 3). These data show no significant difference between isogenic pairs of hybrids in 1997 or 1999.

Agricultural reports show that *Bt* hybrids have significantly higher yields when compared to non-*Bt* plants in contrast to our results. Current estimates of 5% yield loss/borer per plant for first generation ECB and a 3% yield loss/borer per plant for second generation are widely used (Mason et al. 1996, Steffey and Gray 1994). According to Graeber et al. (1999), non-transgenic hybrids showed a decrease in yield of 6.3% with first and second ECB infestation compared to a treatment without infestation. When infested with both ECB generations, the susceptible (non-transgenic) hybrids yielded significantly less (6.6%) than their *Bt* counterparts. A three-year study by Pioneer Seed Company shows that *Bt* hybrids exhibit a 1.6 bushel/hectare yield advantage under low ECB infestation, a 5.2 bushel/hectare advantage under medium infestation, and a 11.6

bushel/hectare yield advantage under high infestation levels compared to hybrids without *Bt* (Olson 2000). These data support *Bt* corn to exhibit higher yields than their non-transgenic counterpart when considering insect infested conditions. Even under heavy infestations in our plots, no significant difference was found between the resistant transgenic and susceptible non-transgenic hybrids. Some reasons for the discrepancies between our data and other studies are possible. One of these reasons could be the size of our plots compared to the larger plot sizes with more replications in the other studies. Smaller plots result in smaller sample sizes, which result in an increased variability. Another reason may be hand harvesting in relation to machine harvesting. Hand harvesting permits all ears to be collected whereas machine harvesting in the other studies may cause broken stalks and detached ears (due to ECB boring) to be missed and seed to be lost. Yet another reason may be the varieties of hybrids used. Our study used only Golden Harvest varieties that express *Bt* trait in all cells throughout the life of the plant whereas other studies used maize varieties expressing *Bt* only in green tissue and pollen or in green leaf tissue with active chlorophyll production offering little late season protection.

Resistant insects are becoming a problem to the agricultural community with the use of genetically modified (GMO) crops. Scientists argue that the overplanting of *Bt* corn will lead to development of ECB resistance to *Bt* toxins (Ostlie et al. 1997). Current research is being conducted by the seed corn industry to avoid insect resistance to *Bt*, which includes development of corn that includes different and multiple killing proteins (Hyde et al. 1999).

Summary

In summary, apart from the heavier seed with Bt hybrids when compared to its isogenic pair, no significant differences were found between the isogenic maize hybrids for most comparisons in the study, whether or not they exhibited the *Bt* trait for resistance to *Ostrinia nubilalis*. For both growing seasons, we found significance with the use of nets/insecticides on the plants whether or not they expressed the *Bt* trait. These plants with the use of nets/insecticides had higher seed totals and kernel mass than those not sprayed. ECB infestation and harvest results on plants do not reflect any one physiological mechanism but may affect several physiological processes. The *Bt* inserted maize hybrids did not show any difference with higher yields, larger seed mass or physiological changes when compared to its isogenic pair not containing *Bt*. A decrease in physiological activity in 1999 was mainly due to drought stress in the early part of the season. This response may have been a variable during seed production in 1999 affecting seed totals and mass. In conclusion, our results provide some insight into the differences in physiological factors, higher seed totals, and mass with the incorporation of the *Bt* bacteria into the corn plant.

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Table 1. Total number and dry weights of seeds harvested for *Bt* transgenic pairs of hybrid maize in 1997 and 1999 at Clinton, IL.

Hybrid Pairs	Seed Totals[1] (per treatment)		Seed Mass[2] (g)	
		1997		
H-2493	4009	a[3]	26.7	b
H-2493*Bt*	3867	a	28.6	a
		1999		
H-2581	8087	a	34.9	a
H-9481*Bt*	7923	a	33.4	a
H-9345	10840	a	27.6	a
999803*Bt*	9989	a	27.9	a

1 In 1997, 10 plants were used per treatment to determine seed totals, and in 1999 all plants were harvested in the treatment.

2 Mass was measured per 100 kernels.

3 Means followed by a different letter within a transgenic pair in a column are significantly different as determined by Duncan's multiple range test, 5% level.

Table 2. Field use of Nets and/or insecticide sprays in the experimental plots for 1997 and 1999 showing seed totals and mass of seed.

Treatment	Seed Totals		Seed Mass (g)	
1997 (H-2493; H-2493*Bt*)				
Nets and Spray (No ECB)	4101	a[1]	27.5	a
No Nets or Spray (ECB)	3775	a	27.8	a
1999 (H-2581; H-9481*Bt*)				
Nets and Spray (No ECB)	9693	a	35.9	a
No Nets or Spray (ECB)	6316	b	32.4	b
1999 (H-9345;999803*Bt*)				
Nets and Spray (No ECB)	11475	a	27.9	a
No Nets or Spray (ECB)	9354	b	27.6	a

[1] Means followed by a different letter within a transgenic pair in a column are significantly different as determined by Duncan's multiple range test, 5% level.

Table 3. Percent germination (1997 or 1999) and root length (1999) for seed from *Bt* transgenic pairs of hybrid maize.

Hybrid Pairs	Germination (%)		Root Length (cm)
	1997		
H-2493	99	a[1]	N/A
H-2493*Bt*	97	a	N/A
	1999		
H-2581	98	a	9.2 a
H-9481*Bt*	95	a	8.4 a
H-9345	96	a	9.1 a
999803*Bt*	97	a	8.4 a

[1] Means followed by a different letter within a transgenic pair in a column are significantly different as determined by Duncan's multiple range test, 5% level.

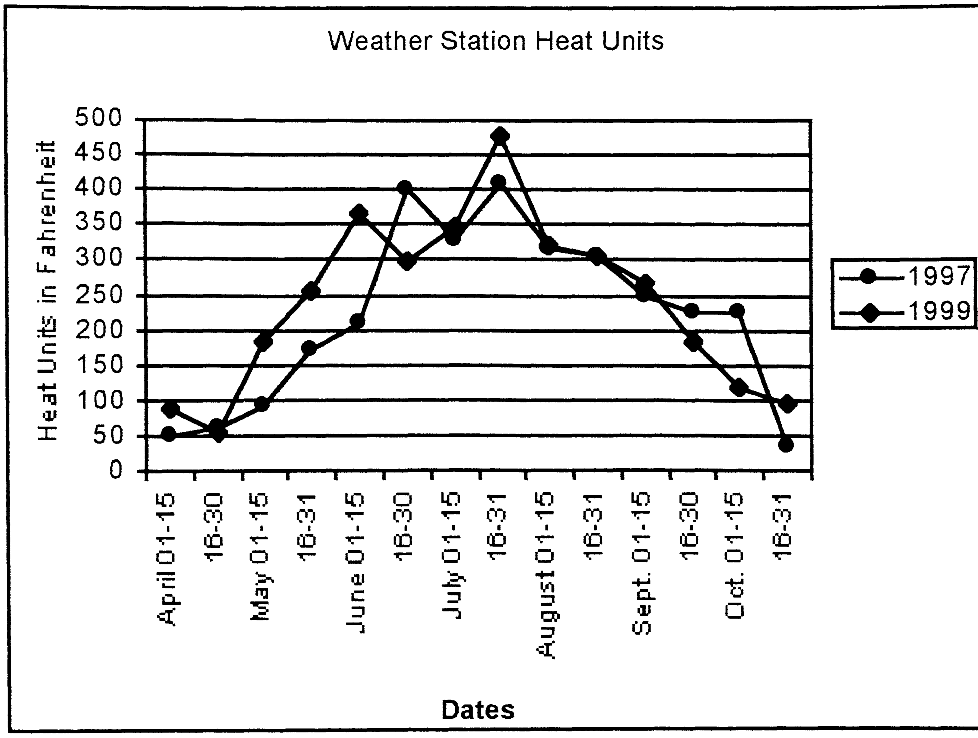

Figure 1. Heat units accumulated between April and October from a weather station located 4 kilometers east of Wapella, IL in 1997 and in Lincoln (until August 31) or Wapella, IL in 1999. Heat units are figured between 50 and 86°F.

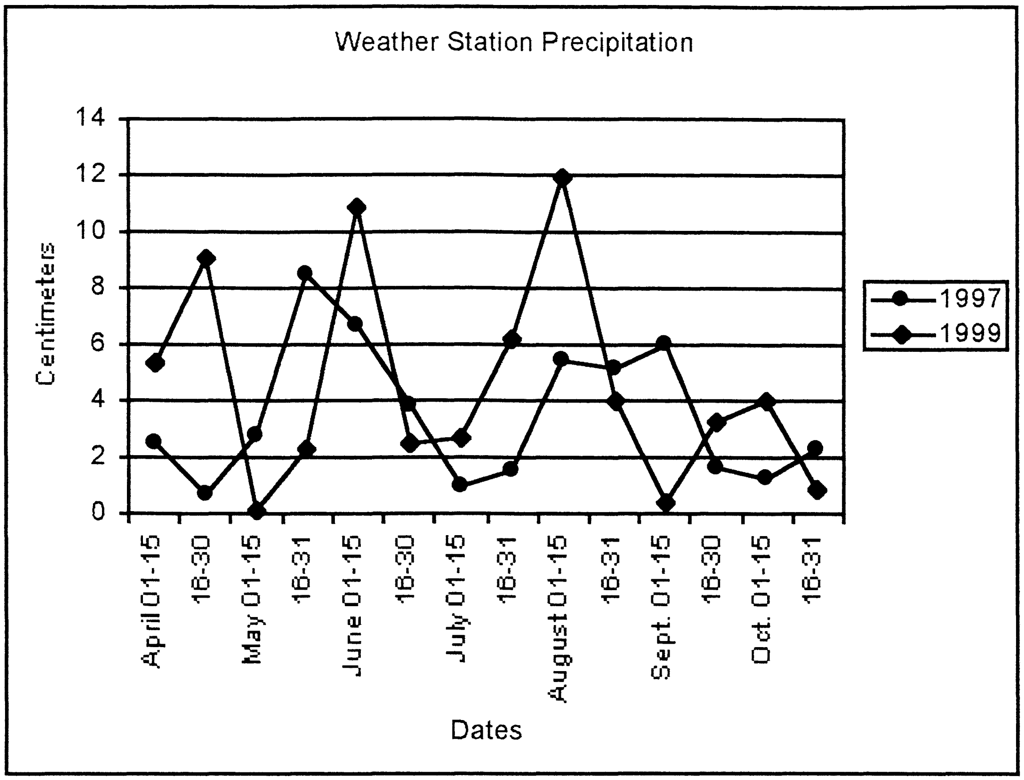

Figure 2. Precipitation between April and October from a weather station located 4 kilometers east of Wapella, IL in 1997 and in Lincoln (until August 31) or Wapella, IL in 1999.

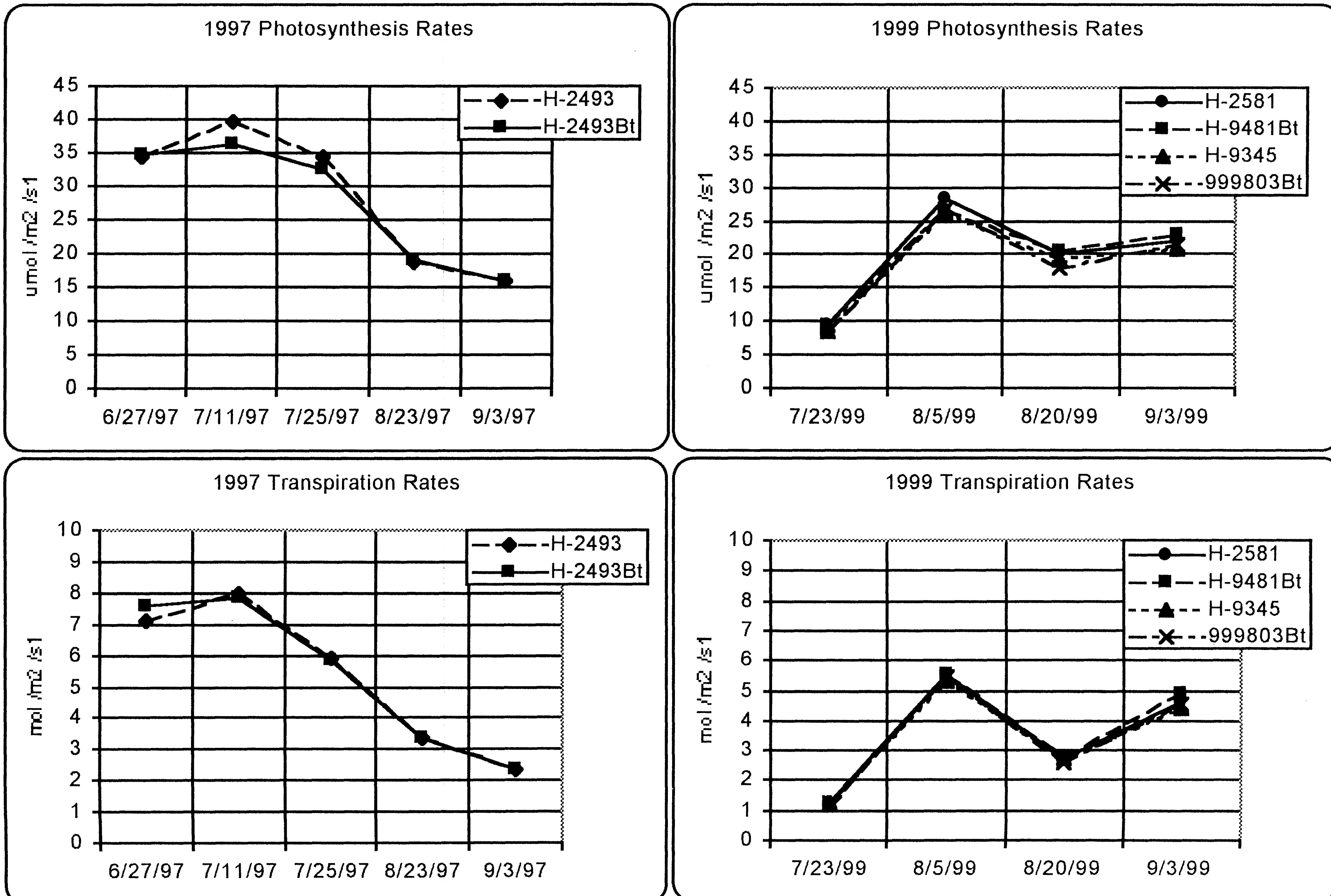

Figure 3. Photosynthesis and transpiration rates of transgenic pairs of corn hybrids over the season in 1997 and 1999 at Clinton, *IL*. No significant differences between associated transgenic pairs. Three transgenic pairs of hybrids are shown: (H2493, H2493*Bt*); (H2581, H9481*Bt*); (H9345, 999803*Bt*). Data lumped for all nets/insecticides and for all ECB infestations due to no significant interactions.